Journal of Forensic and Legal Medicine 19 (2012) 369–372

Contents lists available at SciVerse ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Clinical practice

Interference of tobacco smoke with immunochromatography assay for urinary drug detection

Dhouha Haj Mouhamed PhD [a,b,*], Asma Ezzaher PhD [a,b], Hajer Mabrouk PhD [a,b], Mohamed Hachem Sâadaoui PhD [a,b], Fadoua Neffati PhD [a], Wahiba Douki PhD [a,b], Lotfi Gaha PhD [a,b], Mohamed Fadhel Najjar PhD [a]

[a] Laboratory of Biochemistry-Toxicology, Monastir University Hospital, Monastir 5000, Tunisia
[b] Research Laboratory "Vulnerability to Psychotic disorders LR 05 ES 10", Department of Psychiatry, Monastir University Hospital, Tunisia

ARTICLE INFO

Article history:
Received 25 April 2011
Received in revised form
26 February 2012
Accepted 21 April 2012
Available online 15 May 2012

Keywords:
Tobacco smoke
Interference
Multi-parametric tests
Benzodiazepines

ABSTRACT

Background: This study aimed to evaluate the interference of tobacco smoke on immunochromatography assay of urinary drug detection.
Methods: Our study included 256 voluntary subjects (143 passive smokers and 113 current smokers). Cotinine was measured by immunoenzymatic method and thiocyanates (SCN^-) by selective electrode. Urinary drug was detected by immunochromatography assay. A positive result is completed by an analytical method with an immunometric assay.
Results: False positive results for benzodiazepines are significantly more frequent in smokers compared with passive smokers (90.2% *Vs* 22.4%; $\chi^2 = 116.62$, $p < 10^{-3}$). For smokers, the number of cigarettes was significantly higher in subjects with falsely positive results for benzodiazepines compared with subjects with negative results (32 ± 11 *Vs* 20 ± 10; $p = 0.04$). Between these two groups, we established a significant difference for urinary cotinine (345 ± 211 *Vs* 117 ± 54 μg/μmol; $p < 10^{-3}$) and for plasma SCN^- (101.6 ± 3.4 *Vs* 98.8 ± 2.1 μmol/L; $p = 10^{-3}$). Urinary cotinine and consumption duration present the highest values of areas under curves (AUC) of the receiver-operating-characteristic (ROC) curves. The cut-off of 167.6 μg/μmol and 10.5 years were found as predictive factors of false positive results.
Conclusion: Tobacco smoke interferes with immunochromatography assay of urinary drug detection; therefore, all subjects must be questioned about their smoking status to avoid such false results during results interpretation.

1. Introduction

Numerous compounds such as cannabis and some drugs are known as interferant with the immunochromatography assay drug detection. Urine screening for drugs-of-abuse is used in the evaluation of patients presenting to the emergency department with altered states of consciousness and for pre employment or random employee surveillance programs.[1,2] Samples that produce positive values in these screening assays are usually confirmed by an alternate method such as gas chromatography, high-performance liquid chromatography or, most commonly, gas chromatography–mass spectrometry. This two-tiered approach is designed to minimize the occurrence of false negatives and positives and yet still provide expeditious, presumptive evidence for therapeutic or other intervention. The ideal screening assay produces no false negatives, very few false positives, is inexpensive and readily automated. Several immunoassay methods for the analysis of benzodiazepines in urine and blood/plasma are available and some discrepancies between them have been described.[3,4] They are frequently used as initial test for screening, of plasma or urine for the presence of benzodiazepines. For this reason, the benzodiazepines sensitivity will not only be dependent on the cross-reactivity of the antibodies to the benzodiazepines, but also on the profile of metabolites present in urine, and the amount of target drug.

This study aimed to evaluate the interference of tobacco smoke with immunochromatography assay of urinary drug detection and to investigate the correlation between smoking status (number of cigarettes smoked/day and consumption duration/years) and biological tobacco markers (urinary cotinine and plasma SCN^- levels) with false positive results.

* Corresponding author. Laboratory of Biochemistry-Toxicology, Monastir University Hospital, Monastir 5000, Tunisia. Tel.: +216 73461144; fax: +216 73460678.
E-mail address: hajdhouha@yahoo.fr (D. Haj Mouhamed).

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doi:10.1016/j.jflm.2012.04.010

2. Materials and methods

2.1. Study design

2.1.1. Population

The initial study was conducted with 256 volunteer subjects, 143 were non-smokers (passive smokers) aged 44. 20 ± 15.81 years and 113 were current smokers aged 38.02 ± 17.49 years.

2.1.2. Samples

Urine samples were obtained from volunteer subjects with informed consent from all participants. These samples were either used the same day or frozen at −20 °C until required for analysis. All the samples were analysed for cotinine and detection drug.

Blood samples were drawn from an antecubital vein into vacutainers containing Ethylene Diamine Tetraacetic Acid (EDTA). After centrifugation, aliquots of plasma were frozen at −20 °C until analysed for plasma SCN^-.

2.2. Methods

2.2.1. Smoking questionnaire

Subject information and cigarette smoking outcome data were collected in a structured interview. The available data were limited to the classification of smoking to two categories: former, or current. The majority of current and former smokers were able to provide information on the number of cigarettes they smoked and the duration of smoking. All subjects were questioned about their medical history and individuals taking drugs were excluded from our study.

2.2.2. Laboratory analysis

Cotinine level was determined by immunoenzymatic method (Konelab 30™, Thermo Electron Corporation), and expressed as μg/μmol creatinine in urine. Plasma SCN^- levels were determined by selective electrode (Ionometer Seven Multi™ S80 from Mettler Toledo), and expressed as μmol/L.

Urinary screening drug was performed by immunochromatography assay (DrugCheck™: ALL DIAG Laboratories, 67038 Strasbourg) based on the principle of competitive binding. Every positive result was completed by an immunometric assay on the TDx® (Abbott Laboratories, Abbott Parck, IL 60064) based on fluorescent polarization immunoassay was capable of detecting the more potent benzodiazepines to obtain a confirmed result. A stated cut-off concentration of 200 ng/ml was claimed by the manufacturers.

2.2.3. Statistical analysis

Standard descriptive statistics, correlation coefficients, and significance tests were calculated using the SPSS 17.0. Differences between mean values were evaluated by Student's t test and between frequency values by chi square test (χ^2). A p value of less than 0.05 was considered to represent a statistically significant difference between groups. The influence of the cut-off for two biological markers and smoking status parameters on sensitivity and specificity is illustrated by plotting a ROC curve.

3. Results

In Table 1, we showed that false positive results for benzodiazepines, are significantly more frequent in smokers compared with passive smokers (90. 2% *Vs* 22.4%; $\chi^2 = 116$, $p < 10^{-3}$).

The number of cigarettes smoked was significantly correlated with benzodiazepine levels ($r = 0.172$; $p = 0.05$), determined with immunometric assay on the TDx®, however these levels were under a cut-off detection claimed for this method. Contrary to consumption duration, there was no difference between two groups (Table 2).

Number of cigarettes smoked was significantly higher in subjects with falsely positive results for benzodiazepines compared with subjects with negative results (32 ± 10 *Vs* 17 ± 5; $p < 10^{-3}$), The same result was found for consumption duration (Table 3).

Between these two groups, we established significant difference for urinary cotinine (345 ± 211 *Vs* 117 ± 54 μg/μmol; $p < 10^{-3}$) and for plasma SCN^- concentrations (101.6 ± 3.4 *Vs* 98.8 ± 2.1 μmol/L; $p = 10^{-3}$).

Fig. 1 shows the ROC curves for smoking status parameters (cigarettes smoked/days and consumption duration) and tobacco biological biomarkers (urine cotinine and plasma SCN^-). The areas under these curves are 0.857, 0.823, 0.788 and 0.787 respectively, for urine cotinine, consumption duration, plasma SCN^- and cigarettes smoked/days.

Table 4 shows that specificity of urine cotinine was 80% and sensibility equal to 80%. These two values correspond to a cut-off of 167.60 μg/μmol Cr. Plasma SCN^- presents the same value of specificity and sensibility (67%) correspondent to a cut-off of 99.63 μmol/L. Concerning cigarette smoked/days, the value of specificity and sensibility was respectively 70% and 67% correspondent to a cut-off of 20 cigarettes/days. Finally specificity and sensibility of consumption duration were respectively 83% and 82% correspondent to a cut-off of 10.5 years.

4. Discussion

In this study, we showed false positive results for benzodiazepines by DrugCheck ™ test, which are significantly frequent in smokers compared with passive smokers. This can be explained by the presence of benzodiazepines inverse agonists in tobacco smoke with high affinity with binding sites of anti-benzodiazepines antibody, such as β-carbolines (Fig. 2): norharman and harman were isolated in cigarette smoke and characterized as two potent inhibitors of MAO-A (harman and norharman) and MAO-B (norharman).[5] β-carbolines are heterocyclic pyridoindole alkaloids produced through a Pictet–Spengler condensation from indoleethylamines and carbonylic compounds followed by an oxidation step, are putative endogenous ligands of the benzodiazepines receptor [6,7]. These alkaloids appear endogenously in biological fluids and tissues, including the brain,[8,9] and are also xenobiotics.[10,11]

Various β-carbolines bind at benzodiazepines receptors, and previous structure affinity studies suggest that the presence of a 3-position substituent (e.g. amide, ester, carbinol) and a fully aromatic ring system are optimal for benzodiazepines receptor binding (Fig. 2).[9] Noticeably, β-carboline alkaloids exhibit a wide spectrum of pharmacological and neuro-active actions, bind to several receptors in the brain such as benzodiazepines, imidazoline, and serotonin, and can alter the levels of neurotransmitters.[9,12] This hypothesis was proved by comparing in vivo and in vitro antagonism of benzodiazepines by harman and other β-carbolines.[12]

Table 1
Urinary benzodiazepine detection according to smoking status.

	Current smokers ($n = 113$) (%)	Non-smokers ($n = 143$) (%)	OR [95%CI]
False positives results	102 (90.2)	32 (22.4)	32.16 [15.41, 67.13]
Negatives results	11 (9.8)	111 (77.6)	
χ^2; p		116.62; $p < 0.001$	

Yule Q coefficient = 0.94.

Table 2
Variations of smoking status parameters (cigarettes smoking number and consumption duration) according to benzodiazepines levels (<200 ng/ml) using immunometric assay.

Smoking status	Benzodiazepines levels (ng/ml)		
	<100 ($n = 97$)	[100 − 200] ($n = 5$)	p
Cigarettes smoked/days	28 ± 11	36 ± 6	0.02
Consumption duration/years	13.3 ± 7.6	13.2 ± 3.5	0.93
r Pearson; p		0.172; 0.05	

Table 3
Benzodiazepine detection results according to smoking status, urinary cotinine and plasma SCN^- concentration.

Parameters	False positives results ($n = 102$)	Negatives results ($n = 11$)	p-value
Cigarettes smoked/day	32 ± 10	17 ± 5	0.001
Consumption duration/years	13.5 ± 7.5	6 ± 4	0.002
Urinary cotinine (μg/μmol Cr)	345.23 ± 211.12	116.59 ± 53.92	0.001
Plasma thiocyanates (μmol/L)	101.59 ± 3.40	98.80 ± 2.13	0.009

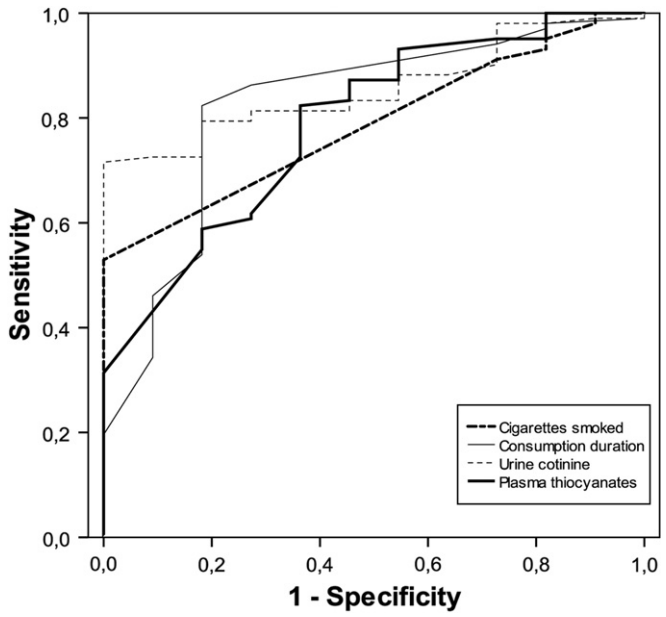

Fig. 1. Receiver-operating-characteristic (ROC) of smoking status parameters and tobacco biological biomarkers.

This interference with some components of tobacco smoke confirms the lack specificity of DrugCheck™ test. Taken together, these results suggest that chromatography assay will be suitable for routine drug screening. However, high-performance liquid chromatography is becoming increasingly the method of choice for simultaneous quantification and confirmation of many polar and/or chemically instable drugs, including benzodiazepines.[13]

The number of cigarettes smoked was significantly correlated with benzodiazepine levels ($r = 0.172$; $p = 0.05$), however these levels were under a cut-off detection claimed for this method.

Cross-reactivity was determined for many compounds (Epinephrine, caffeine, ketamine…). These following compounds show no cross-reactivity when tested with the immunometric assay at a concentration at the 200 ng/ml, but the interference for carbolines should be determined.

In this study, we noted, that the number of cigarettes smoked was significantly higher in subjects with falsely positive results for benzodiazepines compared with subjects with negative results ($p = 0.04$). This finding confirms a positive correlation between β-carbolines (harman and norharman) levels and number of cigarette smoked/day.[5] Tobacco smoke is a relevant source of β-carbolines. Depending on smoking habits, intake may account for up to 1.14 μg/kg for norharman and 0.57 μg/kg for harman when 20 cigarettes are consumed (5). Inhalative exposure was shown to result in rapid and effective uptake of β-carbolines and smoking of cigarettes has been shown to result in a rapid increase of plasma levels of harman and norharman.[14] No data exist on the effects of passive smoking on β-carboline plasma levels but it has been shown that environmental tobacco smoke contains high levels of harman and norharman.[14]

Urinary cotinine and plasma thiocyanate are known as two biological biomarkers of cigarette smoking.

For smokers, we established a significant difference for urinary cotinine ($p = 0.0003$) and for plasma SCN^- concentration ($p = 0.0008$) between subjects with falsely positive results for benzodiazepines compared with subject with negative results. This difference between the two groups was not surprising, because urinary cotinine and plasma SCN^- levels are considered as markers of tobacco smoke exposure.[15]

The areas under ROC curves are 0.857, 0.823, 0.788 and 0.787 respectively, for urine cotinine, consumption duration, plasma SCN^- and cigarettes smoked. Specificity of urine cotinine was 80% and sensibility equal to 80%. These two values correspond to a cut-off of 167.60 μg/μmol. Plasma SCN^- presents the same value of specificity and sensibility (67%) correspondent to a cut-off of 99.63 μmol/L. Concerning cigarette smoked, the value of specificity and sensibility was respectively 70% and 67% correspondent to a cut-off of 20 cigarettes/days. Finally specificity and sensibility of consumption duration were respectively 83% and 82% correspondent to a cut-off of 10.5 years.

Urinary cotinine and consumption duration present the highest values of AUC of the ROC curves. The cut-off of 167.6 μg/μmol and 10.5 years were found as predictive factors of false positive results.

In conclusion, for smokers, some multi-parametric tests of urinary drug detection by immunochromatography were often falsely positive for benzodiazepine; therefore, all subjects must been questioned about their smoking status to avoid these false results. These data allow the development of appropriate methods for the detection of benzodiazepines and their metabolites. The conjunction of the retention properties to the spectral profile

Table 4
Specificity and sensibility of smoking status parameters and tobacco biological biomarkers.

Parameters	AUC [95%CI]	Cut-off	Specificity (%)	Sensibility (%)	p-value
Urine cotinine (μg/μmol Cr)	0.857 [0.78–0.93]	167.60	80	80	0.0001
Consumption duration/years	0.823 [0.68–0.95]	10.5	83	82	0.0001
Plasma thiocyanates (μmol/L)	0.788 [0.65–0.94]	99.63	67	67	0.002
Cigarettes smoked/day	0.787 [0.68–0.89]	20	70	67	0.002

AUC: Area under the curve.

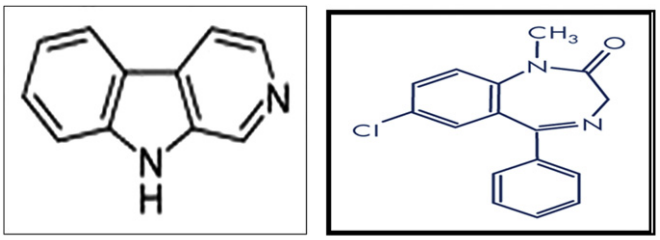

Fig. 2. Chemical structures of carbolines and benzodiazepine.

provided by diode array detection system can be usefully adapted for substances screened.

Ethical approval

None.

Research funding

None declared.

Conflict of interest

The authors stated that there are no conflicts of interest regarding the publication of this article.

Acknowledgements

This research was supported by the research team of the laboratory of Biochemistry-Toxicology, CHU Fattouma Bourguiba, Monastir, Tunisia.

Authors thank Mr. Samir Boukattaya for his assistance and helpful on English language correction.

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